outside of the animal, and wherein the essential gene is a copy of a [native]wild-type gene of the microbial cell; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal,

wherein the [native]wild-type gene is inactivated in the cell,

wherein the cell is a member of the *Enterobacteriaceae*.

Remarks

Claims 1-4, 8-14, 16, 20, 23, 24, 27-32, 35, 37 and 41-44 are pending in this case. Claim 45 has been cancelled and claims 1, 27 and 30 have been amended to more particularly point out and distinctly claim the invention. Support for the amendments is found at least at page 12, lines 23-25.

The withdrawal of all previous claim rejections is noted with appreciation. Discussion regarding the current rejections is provided below.

The claims are directed to (a) microbial cells comprising an Environmentally Limited Viability System that are viable in a permissive environment and non-viable in a non-permissive environment; (b) methods of making the cells; and (c) methods of inducing immunoprotection using the cells. As discussed in the specification at page 11, lines 1-11, a permissive environment is "an environment in which microorganisms incorporating an Environmentally Limited Viability System are viable."

The Environmentally Limited Viability System in the claimed microbial cells comprise at least two genes. These genes are an essential gene and a lethal gene. The essential gene is a copy of a wild-type gene of the microbial cell, wherein the wild-type gene itself is inactivated in the cell. Expression of the essential gene is essential to the viability of the cell and the essential gene is expressed in the permissive environment but not in the non-permissive environment. Thus, by virtue of its essential nature, the lack of expression of the essential gene in the non-permissive environment causes cell non-viability. In contrast to the essential gene, a lethal gene is "lethal to the host microorganism when expressed in the cell." (Specification, page 16, lines 7-8). Expression of the lethal gene is lethal to the claimed cell and the lethal gene is expressed in the non-permissive environment but not in the permissive environment. Thus, by virtue of its lethal nature, the expression of the lethal gene in the non-permissive environment causes cell non-viability.

Based on the above discussion, the essential gene and the lethal gene must necessarily be two different genes. This is because they are expressed in mutually exclusive environments. The essential gene is only expressed in the permissive environment, where the lethal gene is not expressed, and the lethal gene is only expressed in the non-permissive environment, where the essential gene is not

expressed. To put this another way, in the permissive environment, the essential gene is expressed but the lethal gene is not, and in the non-permissive environment, the lethal gene is expressed but the essential gene is not. Because the two genes have opposite requirements for expression and non-expression, they cannot be the same gene. Thus, the claimed microbial cells must have at least one essential gene and one lethal gene.

As discussed extensively in the specification, the Environmentally Limited Viability System is a unique system for assuring that a microbial cell is only viable in a particular environment (the permissive environment). The System in the claimed cell has a built in redundancy to assure that cell will not be viable in the non-permissive environment, because in the non-permissive environment both the expression of the lethal gene and the lack of expression of the essential gene assures that the cell will lack viability. This contrasts with systems where only one regulatable gene causes lack of viability, as taught for example in Molin et al., U.S. Patent No. 5,702,916, which teaches only the expression of a regulatable lethal gene to render the cell non-viable in a non-permissive environment. See further the discussion below relating to the 35 U.S.C. § 102(e) rejection based on the alleged anticipation by Molin et al.

Rejections under the non-statutory doctrine of obviousness-type double patenting

Claims 1-4, 8-14, 16, 20, 23, 24, 27-32, 35, 37 and 41-44 are rejected under the judicially created doctrine of obviousness-type double patenting over U.S. Patent Application Ser. No. 08/761,769. As there are no allowable claims in the instant application at this time, applicants wish to defer responding to this rejection until such time as there are allowable claims.

Rejections under 35 U.S.C. §102

Claims 1-4, 8-14, 20, 23, 24, 27-29 and 37 are rejected under 35 U.S.C. §102(e) as being anticipated by Molin et al., U.S. Patent No. 5,702,916. It is asserted that Molin et al. discloses a biological containment system comprising a cell containing a recombinant DNA molecule that regulatably expresses a cell-killing function in certain environmental conditions. Specifically, the *hok* gene is cited as expressing a cell-killing function when insufficient concentrations of inhibitory sok are present.

Applicants respectfully request reconsideration and withdrawal of this rejection in light of the following remarks.

Applicants first note that all of the rejected claims contain the limitation that the essential gene is a copy of a wild-type gene of the microbial cell, wherein the wild-type gene is inactivated in the cell. This limitation is not taught by the cited Molin et al. patent. Specifically, the Office Action identifies the *sok* gene of Molin et al. as an essential gene within the context of the instant invention.

Applicants contend that this gene is not a wild-type gene of the microbial cell, nor is an inactivated copy of that gene present in the cell, as is required by the rejected claims. In Molin, the *sok* gene is carried on the plasmid R1, in the parB region (Molin et al., column 10, lines 24-49). Molin does not teach that the plasmid-borne *sok* is a copy of a wild-type gene of the host cell, nor does Molin teach the presence of an inactivated *sok* in the cell.

In addition, all of the rejected claims require that the essential gene be expressed when the cell is in a permissive environment, and not expressed when the cell is in a non-permissive environment. Applicants contend that this limitation is not taught by Molin et al. Specifically, the *sok* gene is not described in Molin as being differentially expressed when in permissive versus non-permissive environments. Rather, the cell killing function is differentially expressed by placing a regulatable promoter upstream of the *hok* gene such that the hok mRNA is expressed at a higher level than the sok mRNA when the cell is in a non-permissive environment (see Molin et al., column 11 lines 26-39). Molin et al. teaches a containment system which functions by regulatably controlling expression of a lethal gene, and makes no suggestion of controlling the expression of an essential gene. In all of the descriptions of the containment system in Molin et al., the *sok* gene is expressed regardless of whether the cell is in a permissive or non-permissive environment. See for example, Molin et al. at column 21, line 30 through column 23, line 60, for descriptions of the disclosed plasmids. Note, in particular, that there is no regulatable promoter controlling expression of the *sok* gene found in any of these constructs. Thus, the claim limitation that "the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment" is not taught by Molin et al.

The Office Action points out, at the bottom of page 6 to the top of page 7, that Molin et al. teaches a system wherein the death of the cell occurs due to the expression of the gene encoding the cell killing function as a result of pre-determined environmental conditions. In contrast, in the claimed invention of the instant application, cell death occurs both because of expression of a lethal gene in a non-permissive environment, and because of non-expression of the essential gene in non-permissive environments.

Claims 1, 4, 10-12, 20, 27, and 41-45 are rejected under 35 U.S.C. §102(b) as being anticipated by Curtiss III (*Engineering Organisms For Safety: What Is Necessary?*, The Release Of Genetically-Engineered Micro-Organisms, M. Sussman et al., editors, Academic Press, 7-20 (1988)). It is alleged in the Office Action at page 7 that Curtiss III teaches a system in which the hok gene is the lethal gene and asd is the essential gene.

Applicants first point out that all of the rejected claims contain the limitation that "the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment." This limitation is not taught by Curtiss III. Specifically, the

section at page 12 of Curtiss III that is emphasized by the Office Action suggests the use of a lethal gene such as *hok* in order to limit the vector of the balanced lethal system to the intended host. In that case, the essential gene, *asd*, is expressed in a non-regulatable manner, in order to provide selective pressure to maintain the vector in the host population by causing death of the host in the absence of the vector. In such a system, regulatable expression of the essential gene is not necessary or desirable. The cell killing function is suggested as a means of preventing expression of the vector by unintended hosts. The promoter suggested, therefore, is one that is repressed so as not to express the lethal gene when in the intended host cell. When the plasmid is transferred to an unintended host, however, the promoter causes expression of the lethal gene. The utility of this system does not extend to causing the death of the intended host organism when released from a permissive environment. Instead, such organisms may remain viable and proliferate in unintended environments.

In contrast, the system of the instant invention insures death of the intended host organism when the host organism is released from a specific permissive environment into a non-permissive environment, as well as death of the intended host when the vector is released, and death of unintended hosts when the vector is transferred to them. The cell-killing function of the instant invention is not only triggered by loss of the vector from the host, as it is in the Curtiss III system, but more importantly, by the release of the host organism from the permissive environment. This is accomplished in the instant invention by utilizing as the essential gene a native gene of the host organism, which has been inactivated in the host, under control of a regulatable promoter. Thus, when the host is released into the non-permissive environment, the essential gene is no longer expressed, resulting in the death of the host. In addition, death of the host organism in non-permissive environments is insured by regulatably expressing a lethal gene, such that when the organism is released from the permissive environment, the promoter is up regulated resulting in cell death by expression of the lethal gene product.

In summary, the cited references teach genetically engineered organisms that contain vectors comprising both lethal genes and essential genes. However, neither Curtiss III nor Molin et al. teaches an essential gene that is under the control of a regulatable promoter. In addition, Molin et al. does not even teach an essential gene that is a native gene, nor does it teach the presence of the inactivated wild-type gene in the cell. Therefore, applicants assert that the claims of the instant application are not anticipated.

Based on the discussion presented above, reconsideration and withdrawal of the rejections put forth in the Office Action dated July 25, 2000 is respectfully requested. Applicants believe that the currently pending claims are allowable. If there are any issues yet to be resolved, the Examiner is invited to contact the undersigned attorney.

Respectfully submitted,

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